

Optogenetic Neuromodulation

Challenges Towards a Clinical Implementation

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Abstract

Optogenetic neuromodulation is a novel approach for bidirectional control of neural signals that are cell-type specific and spatiotemporally precise. Using light for the controlling signal avoids fundamental problems that affect electrode probing. Earlier *in vivo* studies have used fiber optics to deliver light in laboratory settings, but this report looks forward to the challenges that a clinical implementation would face. The relationship of volume of tissue activated, light penetration depth, and optical fiber diameter is explored, as well as its sensitivity to other facts including numerical aperture and varying scattering coefficients. Alternatives for the light source are explored with an eye towards implantable feasibility. Finally, this technique is put in context with regard to existing electrode-based deep brain stimulation treatment.

Introduction

Optogenetic neuromodulation describes an innovative technique that involves using optical methods to trigger and/or suppress neuronal action potentials via genetically expressed opsin proteins. Spearheaded by Dr. Karl Deisseroth's lab at Stanford University, this method has created promising new opportunities to better understand the pathology behind the occurrence of neuropsychiatric diseases and uncover potential treatment options.

Opsins are light-sensitive proteins and two microbial opsins have been successfully expressed in neuronal membrane by using lentiviral gene delivery; channelrhodopsin-2 (ChR2) and halorhodopsin (NpHR). When blue light (470nm wavelength) illuminates ChR2, the opsin acts as an open Na^+ channel and features sub-millisecond kinetics [1]. When yellow light (~580nm) illuminates NpHR, the opsin acts like Cl^- pump at millisecond kinetics [2]. The former depolarizes the membrane and can trigger an action potential, the latter hyperpolarizes and can suppresses action potentials.

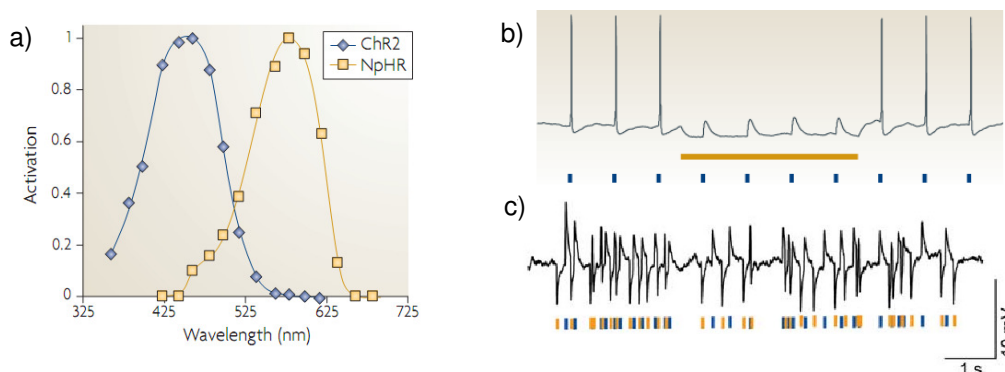


Figure 1. a) Normalized activation spectra for ChR2 and NpHR [3] b) temporally precise triggering and suppression of action potentials [3] c) depolarization and hyperpolarization events from a Poisson light pulse pattern with IPI $\lambda=100\text{ms}$. [2]

Viral delivery of the genes that express the opsins allows researchers to be neuron cell-type specific – a valuable feature because dysfunction of certain kinds of neurons have been associated with specific illnesses. For example, Parkinson's disease has been attributed to the loss of dopaminergic neurons in the substantia nigra pars [3]. Furthermore, the presence of these opsins has no reported side effects on the normal state of the cell [2,4].

This technique has several advantages compared to using traditional electrodes. Electrical stimulation affects all neurons in the vicinity of the electrode, making it difficult to isolate particular neural circuit

behaviors. The genetic expression of opsins into specific cell-types mitigates this problem. The recording of neural activity with electrodes can provide high spatiotemporal resolution, support many simultaneous probe points, be conducted for long periods of time, and can readily be done *in vivo*. However, electrical recording cannot occur simultaneously with electrical stimulation due to interference; the act of stimulation will cause an artifact in the measurement. Simultaneous modulation via optical methods and measurement with electrodes is readily achievable. This is a key advantage because specific neural circuits can now be triggered or suppressed in a reversible manner on millisecond timescales while the effects in the local circuit or in connected neural pathways can be monitored in real-time. Finally, scar tissue that ultimately envelops an electrode tip results in a higher impedance and forces the stimulation current to increase in order to achieve the same result; a move that is bounded by the likelihood of causing further serious cell damage. This optogenetic technique has been explored in several key experiments thus far. The first tests on ChR2 and NpHR were done in cultured neuron populations [2] and later in cortical slices [5]. Successful control of nematode swimming behavior [6], rat whisker movement [7], olfactory sense in mice [8], and rat locomotion [9] has been shown.

This report explores some key issues that will arise when attempting to create a clinical treatment based on the optogenetic neuromodulation technique. The context of this investigation will be with regard to the deep brain stimulation (DBS) treatment currently employed with electrodes to mitigate effects from Parkinson's disease. The optoelectronic components, which consist of the light source and optical fiber, will be evaluated in terms of suitability and practicality. A critical challenge that must be overcome is the validation of the opsin gene delivery in humans, as to whether it is safe and stable for long-term use. Gene delivery is a procedure that is closely monitored and regulated by outside bodies. However, this report will focus on the engineering questions yet to be answered.

Results

Light Delivery

Existing methods for optogenetic neuromodulation utilize optical fibers for light delivery. A fundamental question to ask is how light propagates *in vivo*, and then how does one appropriately size the optical fiber to illuminate a sufficient area? Yaroslavsky *et al.* investigated the optical properties of various human brain tissue (from an autopsy) including the thalamus, pons, white and gray brain matter, and the cerebellum [10]. Using slices 80um to 200um thick and a spot diameter of 2mm, absorption and scattering parameters were measured across a range of wavelengths from 350nm to 1100nm. This study specifically examined scattering coefficients, absorption coefficients, and anisotropy in relation to effective light penetration depth. The optimal wavelength was determined to be the range 1000nm - 1100nm, which is in the infrared spectrum. Note that this is much higher than the two wavelengths used to activate ChR2 and NpHR.

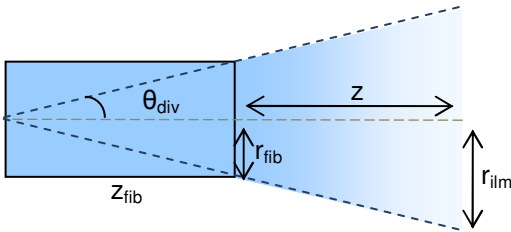
Deisseroth's investigations have focused on light intensity, measured in mW/mm^2 , as a parameter that directly impacts the efficacy of either triggering or suppressing action potentials [7]. Previous studies have found that the minimum acceptable light intensity for ChR2 activation to be $\sim 1\text{mW}/\text{mm}^2$ [7], and two studies with NpHR used intensities of $\sim 10\text{mW}/\text{mm}^2$ [2] and $\sim 21.8\text{mW}/\text{mm}^2$ [6] for activation. Decreasing the light intensity resulted in lower percentages of spikes being inhibited [6]. Campagnola *et al.* further explored localized ChR2 stimulation in brain tissue, utilizing a low-cost blue LED as the light source instead of a diode laser [11]. While it features a smaller physical size, the LED was driven at currents of 1.0A to 1.5A and its uncoupled intensity was 460mW. For a 50um fiber, the intensity at the fiber tip was $32\text{mW}/\text{mm}^2$.

Because a clinical implementation of this technique would most likely utilize the same optical fiber for both wavelengths of light, the intensity should be chosen so that the activation area is equivalent to the inhibition area. This would ensure equal bi-directional control. For an *in vivo* ChR2 study with a rodent motor cortex, a 200um diameter optical fiber was used with a light intensity at the tip being $\sim 380\text{mW}/\text{mm}^2$ [7]. The total transmitted power after passing through 100um of rat cortical tissue dropped by 50%, further dropping to 90% at a depth of 1mm. To gauge the volume of tissue activated, the intensity of light was studied as a function of the transmission fraction and the conical geometry of emitted light at a given

distance from the tip. The transmission factor $T = \frac{1}{Sz+1}$ tracks the loss due to scattering and uses the Kubelka-Munk model where S is the scattering coefficient per unit thickness and z is the thickness of the sample (or depth of light penetration). While some absorption occurs, its coefficient is two to three orders of magnitude below that of scattering [10], so it has been neglected here. The averaged fitted S value for mouse and rat cortical tissue is 10.75mm^{-1} . Next, the geometric decrease was factored in:

$$I(z) = I(z_0) \frac{\rho^2}{(Sz+1)(z+\rho)^2} \text{ with } \rho = r_{fib} \sqrt{\left(\frac{n}{NA}\right)^2 - 1} \quad [7] \text{ with } I(z_0) = \frac{P_{src}}{\pi (r_{fib})^2} \cdot A_f$$

with distance from fiber tip z , intensity at fiber tip z_0 , and the radius of the fiber r . P_{src} is the power available at the source, and A_f is the attenuation factor due to the optical fiber and the imperfect coupling to the light source. A_f was calibrated for use in this report using the reported values of source and output powers with respect to Deisseroth's investigations [7]. The equation above is used to compute the intensity at varying distances from the fiber tip for various fiber diameters, tissue scattering coefficient, and numerical aperture. Next, the volume of tissue activated, the conical region of illumination is calculated for varying fiber diameters and distance from the tip. The divergence half-angle for a multimode optical fiber $\theta_{div} = \sin^{-1}\left(\frac{NA_{fib}}{n_{tis}}\right)$ determines the spread of emitted light, where n_{tis} is the index of refraction of gray matter and NA_{fib} is the numerical aperture of the optical fiber. It is related to the numerical aperture and the index of refraction of the gray matter (1.36) [7].



$$z_{fib} = \frac{r_{fib}}{\tan \theta_{div}} \quad z_{fib} + z \cdot \tan \theta_{div} = r_{ilm}$$

$$Volume = \frac{1}{3} \pi z (r_{ilm}^2 + r_{ilm} r_{fib} + r_{fib}^2)$$

where r_{ilm} is radius of illumination at distance z

Figure 2. Model for conical illumination.

The relationship between illumination intensity and volume of tissue activated for varying distances and optical fiber diameters was computed and is shown below.

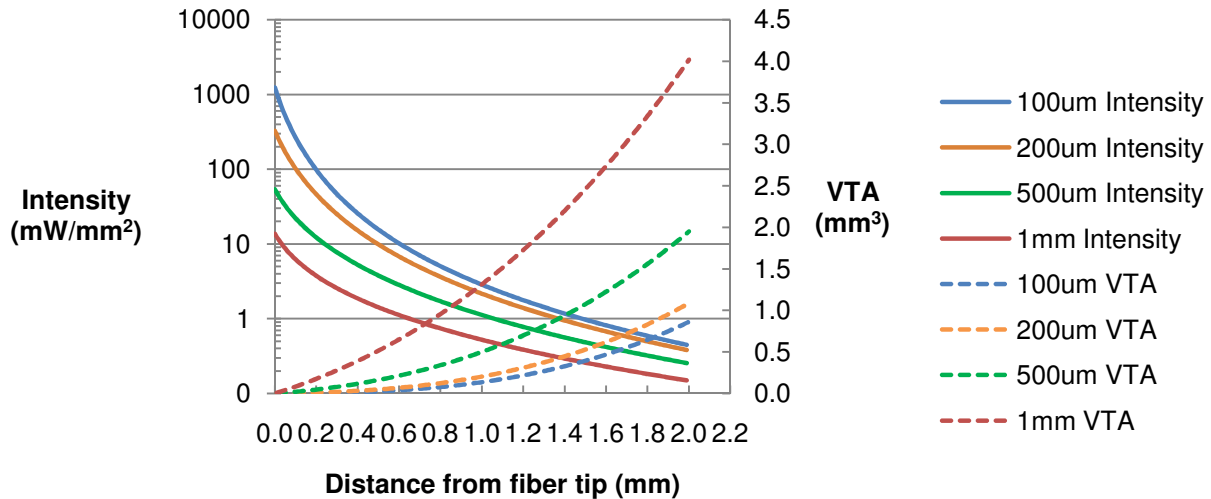


Figure 3. Source power: 20mW, blue light at 473nm for ChR2 activation.

The results shown in Figure 3 for a 200um diameter fiber is confirmed by the findings in [7]. The figure below plots the volume of tissue activated for varying fiber diameters that correlate to the $1\text{mW}/\text{mm}^2$ critical point for light intensity.

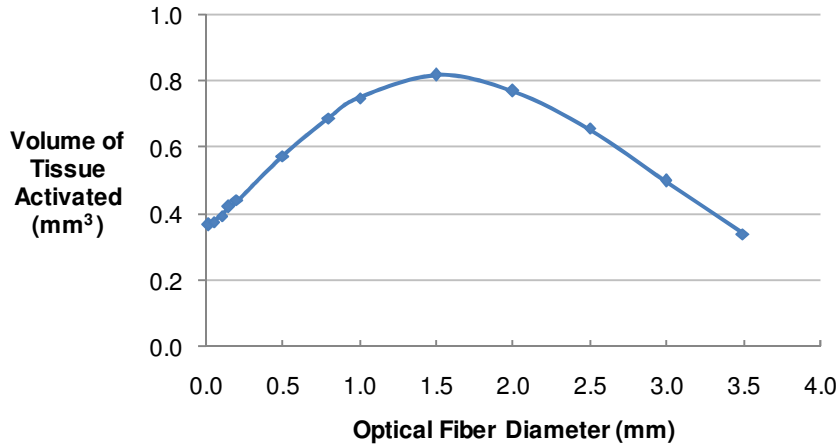


Figure 4. VTA for varying fiber diameters.
 $P_{src} = 20mW$, $NA_{fib} = 0.37$.

For the setup in Figure 4, there is a maximum volume of tissue activated of just over $0.8mm^3$. Varying the power of the light source shows a shift in the optimal fiber diameter for a given power level. As the source power increases, the fiber diameter resulting in the highest tissue volume activated increases. This relationship is not linearly increasing because as the fiber diameter increases, the total volume of tissue that the light is incident upon increases and thus intensity falls.

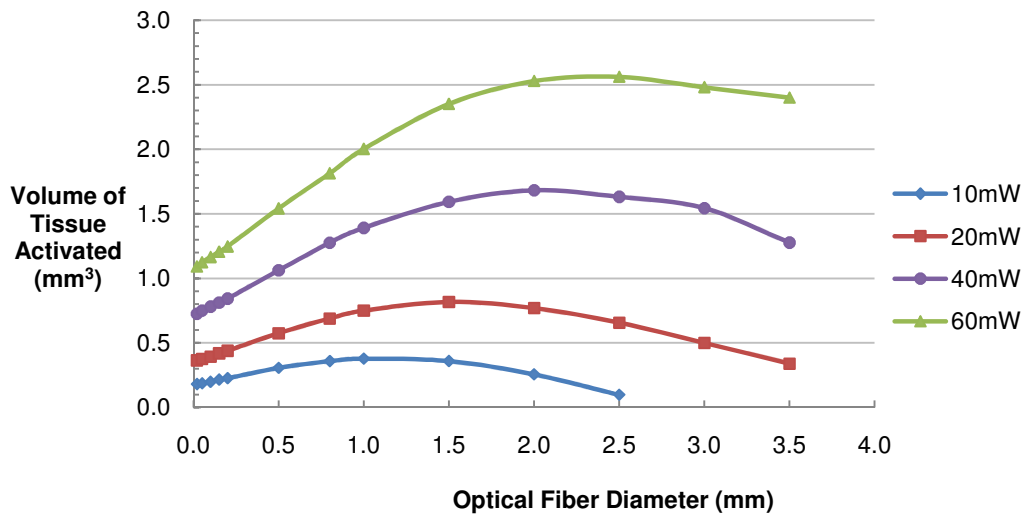


Figure 5. Volume of tissue activated versus fiber diameter for four different power levels.
 $NA_{fib} = 0.37$

Next, the effect of varying the fiber's numerical aperture was explored. ThorLabs, supplier of the optical fiber in [7] also offers multimode fibers with a numerical aperture of 0.22. Figure 6 show a comparison using a 200um diameter fiber and a 20mW power source.

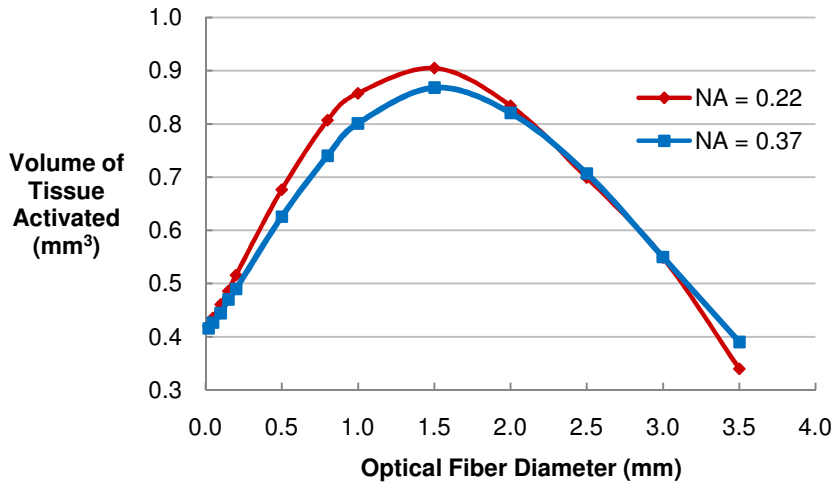


Figure 6. Volume of tissue activated versus fiber diameter for two different numerical apertures. $P_{src} = 20mW$.

The 0.22 numerical aperture optical fiber shows increased tissue volume activation at smaller diameters while being very similar to the 0.37 numerical aperture fiber at large diameters.

Lastly, the figures above use a scattering coefficient S that corresponds to a blue light with a 473nm wavelength. The scattering coefficient decreases with increasing wavelength [10], and the yellow light needed for halorhodopsin activation is at a wavelength of $\sim 580nm$. The decrease of the scattering coefficient on the penetration depth and volume of tissue activation is investigated in Figure 7.

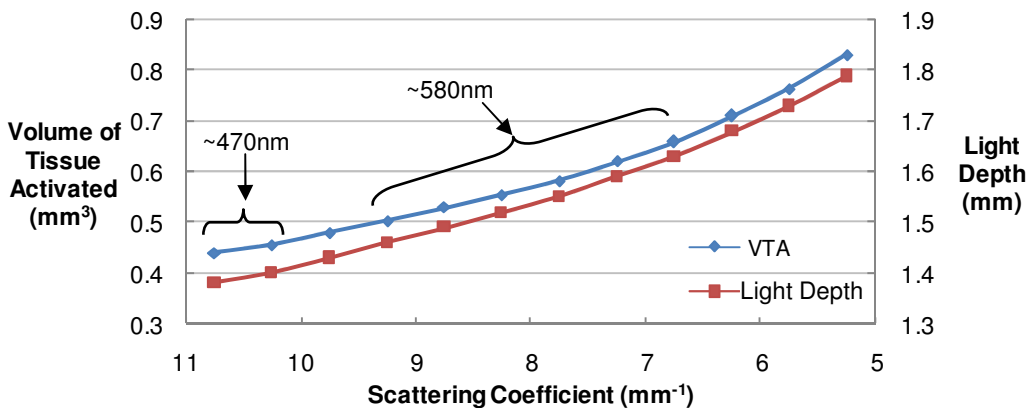


Figure 7. Volume of tissue activated and light penetration depth for varying scattering coefficients. $P_{src} = 20mW$. $NA_{fib} = 0.37$

As expected with decreased scattering, the volume of tissue activated will be higher for yellow light. There is imprecise data available regarding the transmission ratio and scattering coefficient for the $\sim 580nm$ yellow light needed for NpHR activation.

The conical geometry of light output was determined to be a major limiting factor to the volume of tissue activated. Different light output geometries were explored by possible addition of microlenses at the end of the fiber, but no option of merit was found. A spherical lens would have the effect of increasing the divergence angle and thus increasing the volume of tissue directly neighboring the end of the fiber tip. A greater divergence angle would also result in illumination intensity dropping at a higher rate (due to greater surface area of light emitted), but the result would be greater local specificity to the fiber tip.

Light Sources

The diode lasers and LEDs used as light sources in previous investigations were both too large and consumed too much power for subcutaneous implantation. While LEDs are available in smaller physical packages compared to diode lasers, sufficiently powerful blue LEDs still consume on the order of tens of milliwatts to several hundred milliwatts. The high output power requirement is driven by the need to couple to optical fiber, which suffers from attenuation. One alternative is to embed small, low power LEDs

directly in cortical tissue. However, the optical fibers are held in fiber guides, which are small hollow fibers that are held securely to the skull. These fiber guides serve two purposes: a) they serve as a targeted channel for delivering the lentivirals used for transfecting the rhodopsin proteins into the specific neuron cell-types under study, and b) they help hold the optical fiber in position. New robust gene delivery techniques will need to be devised for an approach that involves implanting optoelectronics into tissue. If these components are not anchored, the long term effects of such embedded elements will need to be studied.

Another alternative is to use vertical-cavity surface-emitting lasers (VCSELs) as a light source. A VCSEL array could be fabricated on chip, implanted in a hollowed out portion of the skull [12], and one can readily imagine an array of optical fibers coupled to the laser wells. While this area is deserving of further research, current literature surveyed does not reveal VCSELs operating in the 470nm to 580nm wavelengths needed for ChR2 and NpHR activation. Additionally, the reported a single well output power of only 1 mW [12].

Discussion

Initially this report intended to explore the range of challenges in designing a method of clinically implementing the optogenetic neuromodulation technique. In researching the basic literature and key experiments performed in this field, the issues surrounding light delivery and volume of tissue activated began to dominate focus for two reasons. First, the experiments used different fiber diameters (50um and 200um), different light sources (LED and diode laser), and the relative novelty of halorhodopsin (NpHR) has resulted in a less rigorous analysis of its propagation through brain tissue. One key concern was the use of $\sim 1\text{mW}/\text{mm}^2$ as the minimum light intensity needed to provide satisfactory activation of the rhodopsins. The light intensity versus spike generation/suppression graphs in [6,13] give the impression that intensities close to $\sim 5\text{mW}/\text{mm}^2$ would be preferred. This conservative step would also be appropriate for clinical implementation, and would significantly affect the component parameters. As this technique moves towards using exhibition and inhibition of specific neural circuits to address certain brain disorders, an understanding of the extent to which neural circuits are being addressed will be an important issue. If precisely the same tissue volume is to be sufficiently illuminated for bidirectional control, the source power for the $\sim 580\text{nm}$ yellow light should be scaled down in comparison to the $\sim 470\text{nm}$ blue light. This scale factor can be determined by performing a transmission ratio experiment and fitting the values to the Kubelka-Munk model for blue light in [7].

One finding of interest was an apparent lower bound on the area of illumination that could reliably trigger action potentials with ChR2: a $\sim 100\text{um}$ diameter disk. The large surface area of the neuron's soma would make the density of expressed rhodopsins (and thus switchable ion channels) higher, thus making it easier to depolarize or hyperpolarize the membrane. However, soma are typically on the order of 10um to 50um, depending on the neuron cell-type. Taking a step back, it is recognized single neurons are not likely to be a mission-critical component of a neural circuit. However, perhaps gene delivery or promoter techniques can increase the expression of the rhodopsins to make individual neurons more sensitive and possibly lower the $\sim 1\text{mW}/\text{mm}^2$ minimum intensity threshold needed to reliably trigger action potentials.

Regarding optical fiber sizing, the investigations shown above demonstrate an optimal fiber diameter if the goal is to maximize the volume of tissue activated. This optimal diameter is the result of balancing the light intensity drop due to geometric spreading and tissue scattering in addition to the conical geometry of light output. This optimal diameter increases with increasing source power. The type of characterization performed in this report can be used in other ways. If the goal is to target a neural circuit at a given distance from the fiber tip, the amount of peripheral tissue illuminated can be evaluated or the source power can be adjusted to achieve a desirable combination of parameters. As mentioned briefly earlier, the characterization of NpHR with regard to light intensity sensitivity and optical transmission properties needs to be explored in greater depth. For laboratory experiments, achieving output intensities at the fiber tip several orders of magnitude above the minimum threshold is readily possible.

The chief question posited at the beginning was how the optogenetic neuromodulation technique compares with electrode-based deep brain stimulation treatment. The scar tissue made of glial cells that forms around existing electrodes increases the electrical impedance and requires elevated power delivery

to achieve the same effect. Glial cells are mostly transparent, thus enabling optical control methods to avoid that side effect [7]. Another concern regarding the electrode approach is that changes in capacitance, resistance, and anode/cathode spacing can change the VTA [14-15]. The optical approach is more straightforward because the geometry of the light output can be more readily predicted, though changes in tissue such as coagulation can impact light transmission. A typical DBS electrode is 1.5mm long and 1.27mm in diameter (Medtronic 3387/3389 quadripolar DBS), a diameter that is much larger than current experiments with optical fibers. At typical DBS stimulation parameters of -1V, 90us, and 130 Hz, finite element modeling of the VTA from the Medtronic probe stimulating the VIM region of the thalamus was 57mm³. Even to first-order, this volume is at least one order of magnitude above what is readily capable of optical techniques. It is not known which specific regions of basal ganglia or substantia nigra pars (for Parkinson's disease) is responsible for the illness or their corresponding size. Indeed, one of the hopes for the optical modulation technique is to leverage its spatiotemporal and cell-type specificity to aid in better understanding which circuit malfunctions result in disease. If greater VTAs are needed, multiple optical fibers can be deployed in specific geometries to enable targeting specific volumes and shapes. Finally, the frequencies used with typical DBS electrodes can go to ~150 Hz; the exact frequency is often determined by trial and error with a particular patient. Initial tests with ChR2 and NpHR show that beyond ~30 Hz, reliable depolarization and hyperpolarization is difficult to achieve, regardless of light intensity [2,6,13]. However, in pyramidal neurons the rate achievable through optical methods closely matched that obtained through injection of large currents, indicating that for certain neurons, the method might be limited by the fundamental properties of the neuron [13]. More study is needed with the specific neural circuits under review.

Conclusion

Optical control of neural signals in the brain by gene delivery of rhodopsins into neurons constitutes a revolutionary approach to probing the function and connectivity of the neural circuits. The method offers bidirectional control on millisecond-timescales and can be made cell-type specific. Regarding light delivery, future implementations will be limited by power, size, and thermal constraints. A method of optimizing the diameter of the optical fiber based on desired light penetration depth and volume of tissue activated was explored, as well as how changes in source power and numerical aperture affect VTA. While the blue light for ChR2 activation was well documented, a notable lack of detail regarding the transmission and scattering properties of yellow light was seen and so the predicted decrease in light scattering was explored as to its effects on VTA. A brief survey of available light sources found no such sources currently optimal for implantation. Further work must be done regarding blue LEDs or VCSELs as promising options for the future. Finally, a comparison of the electrode-based DBS treatment option found that the optical method has advantages in avoiding scar tissue effects and more reliable VTA. However, the total VTA stimulated with an electrode is much greater.

Optogenetic neuromodulation is an exciting development in neuroscience that will benefit from the ongoing improvements in optoelectronics and the additional understanding the technique will enable neuroscientists to gain regarding the precise pathology for neuropsychiatric illnesses.

References

- [1] Nagel G, Szellas T, Huhn Wolfram, Kateriya S, Adeishvili N, Berthold P, Ollig D, Hegemann P, and Bamberg E. *Channelrhodopsin-2, a directly light-gated cation-selective membrane channel*. Proc Natl Acad Sci USA. 2003 Nov 25; 100(24): 13940-13945.
- [2] Boyden E S, Han X. *Multiple-Color Optical Activation, Silencing, and Resynchronization of Neural Activity, with Single-Spike Temporal Resolution*. PLoS. March 2007, Issue 3, e299.
- [3] Zhang F, Aravanis AM, Adamantidis A, de Lecea L, Deisseroth K. *Circuit-breakers: optical technologies for probing neural signals and systems*. Nat. Rev. Neurosci. 2007 Aug;8(8):577-81.
- [4] Boyden ES, Zhang F, Bamberg E, Nagel G and Karl Deisseroth. *Millisecond-timescale, genetically targeted optical control of neural activity*. Nat. Neurosci. 2005 Sept; Vol. 8, No. 9. 1263-8.
- [5] Zhang F, Wang L-P, Boyden ES, Deisseroth K. *Channelrhodopsin-2 and optical control of excitable cells*. Nat. Methods. 2006 Oct. Vol. 3, No.10. 785-792.
- [6] Zhang F, Wang L-P, Brauner M, Liewald J, Kay K., Watzke N, Wood P, Bamberg E, Nagel G, Gottschalk A and Deisseroth K. *Multimodal fast optical interrogation of neural circuitry*. Nature. 5 April 2007. Vol. 446. 633-639.
- [7] Aravanis A, Wang LP, Zhang F, Meltzer L, Mogri M, Schneider MB, Deisseroth K. *An optical neural interface: in vivo control of rodent motor cortex with integrated fiberoptic and optogenetic technology*. J. Neural Eng. 2007 Sept; 4:S143-S156.
- [8] B. Arenkiel, J. Peca, I. Davison, C. Feliciano, K. Deisseroth, G. Augustine, M. Ehlers, G. Feng. *In Vivo Light-Induced Activation of Neural Circuitry in Transgenic Mice Expressing Channelrhodopsin-2*. Neuron, Volume 54, Issue 2, Pages 205-218.
- [9] Gradinaru V, Thompson KR, Zhang F, Mogri M, Kay K, Schneider MB, Deisseroth K. *Targeting and readout strategies for fast optical neural control in vitro and in vivo*. J Neurosci. 2007 Dec 26;27(52):14231-8.
- [10] Yaroslavsky AN, Schulze PC, Yaroslavsky IV, Schober R, Ulrich F and Schwarzmaier H-J. *Optical properties of selected native and coagulated human brain tissues in vitro in the visible and near infrared spectral range*. Phys. Med. Biol. 47 (2002) 2059-2073.
- [11] Campagnola L, Wang H, Zylka M. *Fiber-coupled light-emitting diode for localized photostimulation of neurons expressing channelrhodopsin-2*. J. Neuro. Methods. 169 (2008) 27-33.
- [12] Levi O, Lee T, Lee M, Smith S, Harris J. *Integrated semiconductor optical sensors for cellular and neural imaging*. Appl. Optics. 1 Apr 2007. Vol. 46, No. 10.
- [13] Wang H, Peca J, Matsuzaki M, Noguchi J, Qiu L, Wang D, Zhang F, Boyden E, Deisseroth K, Kasai H, Hall W, Feng G, Augustine G. *High-speed mapping of synaptic connectivity using photostimulation in Channelrhodopsin-2 transgenic mice*. PNAS. 8 May 2007. Vol. 104, No. 19. 8143-8148.
- [14] Butson C, McIntyre C. *Tissue and electrode capacitance reduce neural activation volumes during deep brain stimulation*. Clinical Neurophysiology. 116 (2005) 2490-2500.
- [15] Buston C, McIntyre C. *Role of electrode design on the volume of tissue activated during deep brain stimulation*. J. Neural Eng. 3 (2006) 1-8.